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TI Steroid hormone receptor status defines the ***MMTV*** ***promoter*** chromatin structure in vivo.
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TI A novel expression vector composed of a regulatory element of the human leukosialin-encoding gene in different types of mammalian cells.
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Journal code: FOP. ISSN: 0378-1119.

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AU Petitclerc D; Attal J; Theron M C; Bearzotti M; Bolifraud P; Kann G; Stinnakre M G; Pointu H; Puissant C; Houdebine L M
TI The effect of various introns and transcription terminators on the efficiency of expression vectors in various cultured cell lines and in the mammary gland of transgenic mice.
SO JOURNAL OF BIOTECHNOLOGY, (1995 Jun 21) 40 (3) 169-78.
Journal code: AL6. ISSN: 0168-1656.

L5 ANSWER 15 OF 47 MEDLINE DUPLICATE 10
AU Wilson S E; Weng J; Blair S; He Y G; Lloyd S
TI Expression of E6/E7 or SV40 large T antigen-coding oncogenes in human corneal endothelial cells indicates regulated high-proliferative capacity.
SO INVESTIGATIVE OPHTHALMOLOGY AND VISUAL SCIENCE, (1995 Jan) 36 (1) 32-40.
Journal code: GWI. ISSN: 0146-0404.

L5 ANSWER 17 OF 47 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 12
AU Pendse, Girish J.; Bailey, James E.
TI Effect of Vitreoscilla hemoglobin expression on growth and specific tissue plasminogen activator productivity in recombinant Chinese hamster ovary cells
SO Biotechnol. Bioeng. (1994), 44(11), 1367-70
CODEN: BIBIAU; ISSN: 0006-3592

L5 ANSWER 18 OF 47 MEDLINE DUPLICATE 13
AU Archer T K; Zaniewski E; Moyer M L; Nordeen S K
TI The differential capacity of glucocorticoids and progestins to alter chromatin structure and induce gene ***expression*** in ***human*** breast cancer ***cells*** .
SO MOLECULAR ENDOCRINOLOGY, (1994 Sep) 8 (9) 1154-62.
Journal code: NGZ. ISSN: 0888-8809.

Gene, 160 (1995) 283–286
 0378-1119/95/$09.50

GENE 08924

A novel expression vector composed of a regulatory element of the human leukosialin-encoding gene in different types of mammalian cells

(Cloning; transfection; tissue-specific expression; gene therapy)

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SUMMARY

The regulatory element (*RE*) of the human leukosialin (LS)-encoding gene, that encodes a major sialoglycoprotein of human leukocyte and platelet membranes, was used to develop a novel expression vector, pKX. The vector was constructed by cloning a *RE* fragment and the SV40 fragment containing polyadenylation and splicing signals between *Hin*dIII and *Bam*HI sites of the pCAT-Basic vector. The transcription level controlled by this vector was evaluated in six different cell lines using a transient expression assay of chloramphenicol acetyltransferase (CAT). The CAT activity of the pKX vector was compared to the other common expression vectors, namely pMSG (driven by the mouse mammary tumor virus LTR), pcDL-SRα (SV40 promoter/enhancer and HTLV-I LTR), pcDNAI (cytomegalovirus promoter/enhancer) and pCAT-Control (SV40 promoter/enhancer). The level of expression provided by the pKX vector was comparable to that observed with pcDNAI and pcDL-SRα vectors. In different mammalian cell lines, the highest efficiency of expression of the pKX vector was observed in the human T-cell lines, Jurkat and CEM, although the expression of pcDL-SRα-CAT in those cell lines was in the same range. The expression of the pKX vector driven by a non-viral promoter and/or enhancer can be as efficient as that driven by a viral promoter and/or enhancer. Potential uses of this vector may be found in studies of transient gene expression in hematopoietic cells and for gene therapy, particularly the ones involving T-cells.

INTRODUCTION

Expression vectors, designed for the most efficient expression of a given gene, are important in the in vitro and in vivo applications of molecular biology. Early expression vectors were made from SV40 promoters (Templeton and Eckhart, 1984; Lusky and Botchan, 1981), mouse mammary tumor virus (MMTV) LTR (Alton and Vapnek, 1979; Lee et al., 1981; Mulligan and Berg, 1981) and cytomegalovirus (CMV) promoter and enhancer (Seed, 1987; Seed and Aruffo, 1987). Expression vectors of pcD series were constructed using human T-cell leukemia virus I (HTLV-I) LTR (Okayama and Berg, 1982; 1983). Combining the SV40 early promoter and HTLV-I LTR into a new SRα promoter, Takebe et al. (1988) constructed pSRα serial vectors that achieved higher levels of expression than other vectors. Recombinant adenovirus containing the viral major late promoter has been used as the expres-

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Abbreviations: CAT, Cm acetyltransferase; *cat* (*Cat*), gene encoding CAT; CHO, chinese hamster ovary; Cm, chloramphenicol; CMV, cytomegalovirus; FCS, fetal calf serum; HTLV-I, human T-cell leukemia virus I; LS, leukosialin; *LS*, gene encoding LS; LTR, long terminal repeat(s); MMTV, mouse mammary tumor virus; nt, nucleotide(s); *RE*, regulatory element; SRα, SV40 early promoter and HTLV-I LTR; SV40, simian virus 40.

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sion vector for certain genes (Stratford-Perricaudet et al., 1990; Lemarchand et al., 1992).

All these expression vectors are based on virus-derived promoters and lack tissue specificity. In addition the integration of retroviral vectors into host genome suggests at least theoretical risks associated with virus-derived sequences in gene therapy. Other high-efficiency expression vectors with some degree of tissue specificity should be useful. Based on earlier studies (Kudo and Fukuda, 1991), we used an *LS* promoter to construct a highly efficient expression vector in different types of mammalian cells.

EXPERIMENTAL AND DISCUSSION

(a) Construction of expression vectors with the *LS* promoter

Kudo and Fukuda (1991) found that *LS* genomic sequence from nt −91 to +90 exhibited strong promoter activity in a series of *cat* constructs containing various 5′-regulatory regions. The transcription activity is higher than that of CMV promoter and enhancer, one of the strongest promoters in mammalian cells (Boshart et al., 1985; Seed, 1987). In addition, the intron sequence at nt +72 to +439 doubled the transcriptional activity. Therefore we attempted to construct the expression vector using *LS* genomic sequence from nt −91 to +439, as described in the legend to Fig. 1.

(b) Comparison of six mammalian expression vectors

The vectors compared in Fig. 2 have different promoters, pcDNAI-CAT vector (Seed, 1987) has the CMV promoter/enhancer on the 5′-end of *cat* gene, whereas pcDL-SRα-CAT vector (Takebe et al., 1988) contains SRα promoter composed of SV40 promoter/enhancer and HTLV-I LTR. The pCAT-Control vector has SV40 promoter and enhancer at 5′-end of the *cat* gene; but pMSG-CAT has MMTV-LTR, which requires induction with dexamethasone for the activation of its promoter. Our newly constructed pKX-*cat* has the *LS* promoter and enhancer sequence from intron of the *LS* gene (Kudo and Fukuda, 1991) upstream from the *cat* gene. pCAT-Basic, which was the negative control for the study, has no promoter at the 5′-end of the *cat* gene.

(c) Predominant expression of pKX-*cat* in T-cells

The expression efficiency of pKX-*cat* was compared with that of other vectors in transient expression systems (Table I). The efficiency of pcDL-SRα-CAT was in the highest range in all kind cell types studied; it was slightly, perhaps not significantly lower than pcDNAI-CAT in CHO cells and than pKX-*cat* in Jurkat and CEM cells. The high efficiency of pKX-*cat* in the two human T cell lines (Jurkat and CEM) was not observed in CHO, HeLa, K562, and Raji cells in which the efficiency ranged from 30 to 50% of the CAT activity. No expression was observed for pCAT-Basic which lacks a promoter or for pMSG-CAT which requires steroid induction.

In summary, our observations on pcDL-SRα-CAT confirm those of Takebe et al. (1988) that this is an excel-

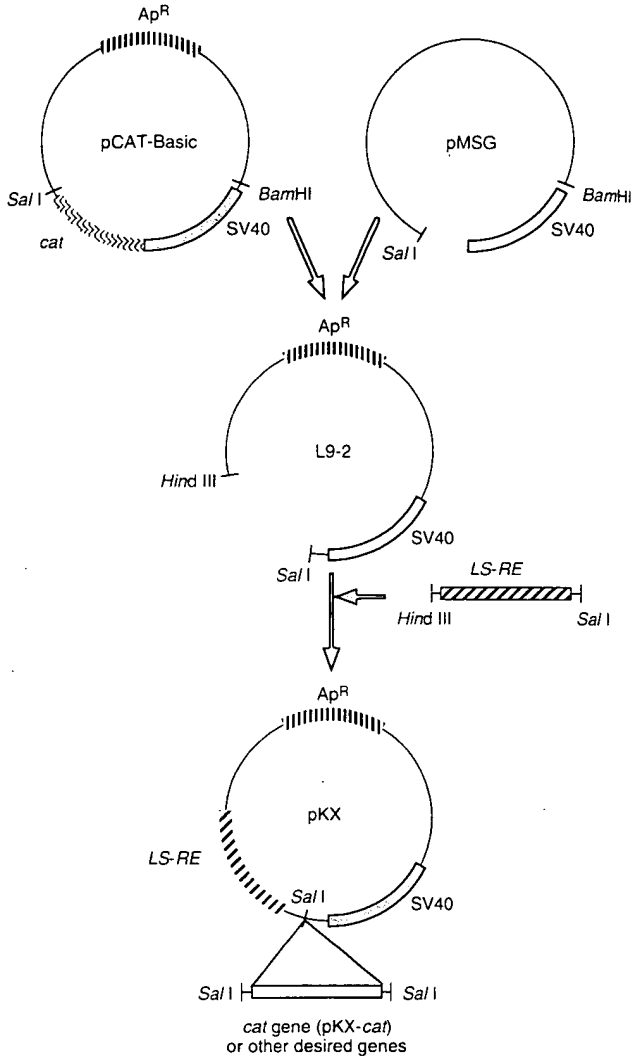

Fig. 1. Schematic diagram of the construction of expression vector pKX-*cat*. The vector pCAT-Basic (Promega, Madison, WI, USA) was digested with *Sal*I + *Bam*HI, and the digested fragment was replaced by the SV40 fragment containing splicing and polyadenylation signals cut from pMSG-CAT (Pharmacia) to form L9-2 plasmid. Two synthetic oligodeoxynucleotides, ST-1 (5′-TTTAAGCTTGGGAGCAGGCG-GGTGGGGCAGGAT) and ANM (5′-TTTGTCGACGGCAGCC-GGAGAAGCAGAACACGC), which contained the sequences from nt −91 to −68 and from +416 to +439 of *LS* gene (Kudo and Fukuda, 1991), and *Hin*dIII and *Sal*I recognition sequences (underlined), respectively, were used as PCR primers. The PCR-amplified DNA fragment containing the *LS RE* was digested with *Sal*I + *Hin*dIII and subcloned between the *Sal*I and *Hin*dIII sites of the plasmid L9-2 to form pKX vector. Finally, pKX-*cat* was constructed by cloning *cat* gene into the *Sal*I site of pKX vector. The pKX-*cat* was sequenced at each joining region of various DNA fragments used for the construction of this vector. Ap^R, ampicillin-resistance marker.

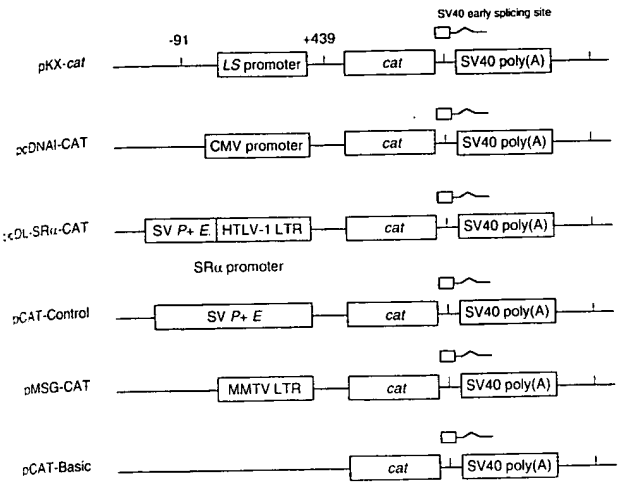

Fig. 2. Comparison of pKX-*cat* with other expression vectors. The difference between the pKX-*cat* and other expression vectors lies in the transcription regulatory elements at 5'-end of each expression vector. pCAT-Basic and pCAT-Control (Promega), pMSG-CAT (Pharmacia) and pcDNA I (Invitrogen) are from commercial suppliers (indicated in parentheses).

lent expression system. Our vector pKX-*cat*, driven by the *LS* promoter and enhancer, has an efficiency comparable to that of pcDL-SRα-CAT in human T-cell lines, and is expressed less efficiently in some other cell lines. The absence of a viral promoter in pKX-*cat* and its expression in a number of cell types may make it a superior expression vector at least for T-cells.

(d) Transcriptional regulation of *LS*-promoter-controlled expression

Leukosialin (CD43) is a highly glycosylated protein found on the surface of T-lymphocytes, granulocytes, monocytes, platelets and hematopoietic stem cells (Dyer and Hunt, 1981; Carlsson and Fukuda, 1986; Gulley et al., 1988; Vargas-Cortes et al., 1988). The expression of *LS* is apparently correlated with the cell lineages and functions. The transcriptional regulatory region of the *LS* gene was characterized recently and was found to have no typical TATA or CAAT boxes but to contain a G-rich segment (Kudo and Fukuda, 1991). In this region, a 14 nt sequence located at nt −53 to −40 was essential to the promoter function in LS-producing cells. Later studies (Kudo and Fukuda, 1994) revealed that the Sp1 transcription factor could bind to this segment and up-regulate the *LS* promoter activity through binding to the 5'-GGGTGG motif, present in the *LS* promoter. Thus the Sp1 factor is important in activating the *LS* promoter although additional factors might be involved in the tissue-specific gene expression. The presence of the Sp1 factor in a wide range of cell types (Gustafson and Kedes, 1989; Sartorelli et al., 1990; Gong et al., 1991; Yu et al., 1991; Robidoux et al., 1992; Kudo and Fukuda, 1994) could explain the expression capacity of the *LS* promoter-controlled expression system in many cell types. The role of Sp1 factor as the molecular mechanism for the expression capacity of pKX was not studied. The highest expression ability in Jurkat and CEM cells may require additional regulatory mechanisms specific in T-cells.

TABLE I

Comparison of CAT activities of pKX-*cat* with four other expression vectors in six cell lines[a]

Vector[b] (promoter)	CAT activity (%)[c]					
	CHO	HeLa	K562	Raji	Jurkat	CEM
pKX-*cat* (*LS* promoter)	41.4	29.1	36.8	51.1	62.6	88.1
pCAT-Basic (no promoter)	1.1	1.1	0.6	0.3	4.5	0.2
pMSG-CAT (MMT-LTR)	1.2	0.6	0.3	3.1	nd	0.3
pCAT-Control (SV40 promoter)	18.6	2.3	2.1	5.5	nd	0.3
pcDNAI-CAT (CMV promoter)	90.7	31.2	18.8	31.3	45.8	10.3
pcDL-SRα-CAT (SRα promoter)	89.1	99.0	55.6	97.3	57.6	86.2

[a] Cultures of human leukemic T-cell lines Jurkat and CEM, B-cell line Raji, erythroid cell line K562, HeLa and CHO cells were described previously (Kudo and Fukuda, 1994). For CHO and HeLa cells, Lipofectin (Bethesda Research Laboratory; Felgner et al., 1987) was used. Jurkat and K562 cells were transfected with Ca-phosphate (Graham and Van der Eb, 1973) using CellPhect Transfection Kit (Pharmacia). Transfection of vector DNA into Raji and CEM cells was carried out by DEAE-dextran method (Grosschedl and Baltimore, 1985) using CellPhect Transfection Kit.

[b] See Fig. 2. Vector DNAs were purified by CsCl gradient before use.

[c] Two days after transfection, cells were harvested by washing from Petri dishes and centrifugation. CHO and HeLa cells were trypsinized before the collection of cells. The cells were lysed by three freeze-thaw cycles after the cell pellet was resuspended in 100 µl 250 mM Tris (pH 7.4). Cell lysates were heated at 65°C for 5 min and supernatant was collected by centrifugation. The CAT activity was assayed by incubating the supernatant of cell lysates in 250 mM Tris (pH 7.4)/4 mM acetyl coenzyme A (Calbiochem)/1 µl of [^{14}C]Cm as described (Gorman et al., 1982). The acetylated compounds were separated from [^{14}C]Cm by thin-layer chromatography (95% chloroform/5% methanol, v/v) on silica gel. After the silica gel was dried, autoradiography was carried out by exposing the silica gel to X-ray Film (Kodak) overnight. CAT activity was calculated by the formula by which the sum of the cpm for the acetylated [^{14}C]Cm is divided by total cpm of [^{14}C]Cm. The spots containing acetylated or unacetylated compounds were cut out and ^{14}C was quantified by scintillation counting. Spots on the X-ray film were quantified by densitometry for the calculation of percentage of acetylation. n.d., not done.

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